REMARKS

Claims 1-53 are pending in the application. Claims 33-51 and 53 are withdrawn from consideration. Claims 1-17, 21, 27 and 52 have been amended to better clarify what Applicants regard as the invention. No new matter has been added by way of this amendment. Thus, as a result of the foregoing amendment, claims 1-32 and 52 remain under consideration. Support for the amendments can be found throughout the specification and particularly on page 15, lines 12-15; on page 16, lines 13-19; on page 17, lines 2-5; on page 18, lines 9-17 and on page 23, lines 17-22, continuing on to page 24, line 1. As a result of the amendments, claims 1-32 and 52 remain under consideration. Reconsideration of this application is respectfully requested.

Claims 1-16 and 52 have been rejected under 35 U.S.C. §101. The Examiner alleges that the claimed invention is drawn to non-statutory subject matter. In particular, the claims recite genetically modified mammals, thereby including humans, which are not patentable. Applicants have amended the claims to recite genetically modified non-human mammals, thereby obviating the Examiner's rejection.

Claims 1-17, 26-27, 31-32 and 52 are rejected under 35 U.S.C. §112, first paragraph for lack of enablement. Applicants respectfully traverse the Examiner's rejection, and have amended the claims to recite genetically modified non-human mammals. Support for enablement of the claims as amended can be found in Example 2, on page 23. Further support for enablement of other non-human mammals in which to practice the present invention is provided in the references included herein for the convenience of the Examiner. Thus, withdrawal of the rejection under 35 U.S.C. §112, first paragraph is respectfully requested.

Claims 1, 10, 11, 15-17, 26-27 and 31-32 have been rejected under 35 U.S.C. §112, first paragraph for not fulfilling the written description requirement. Applicants respectfully traverse the Examiner's rejection and have also amended the claims to better clarify the invention. Support for the amendments can be found on page 23, lines 13-22, continuing on to page 24, line 1; page 15, lines 12-15; and on page 17, lines 2-5. Thus, Applicants respectfully request withdrawal of the rejection.

Claims 1-32 have been rejected under 35 U.S.C. §112, second paragraph as being

indefinite. The claims have been amended to better clarify what Applicants regard as the invention. Withdrawal of the rejection is respectfully requested.

Claims 17-19 and 22-25 have been rejected under 35 U.S.C. §102(b) as being anticipated by Fell et al. (U.S. patent No. 5,202,238). Applicants have amended the claims as noted above and have provided support for the amendments. Applicants have also provided evidence as to the differences between the Fell reference and the instant application. Applicants believe that the amendments and arguments provided to Examiner obviate this rejection. Thus, Applicants respectfully request withdrawal of this rejection.

Claims 20-21 and 27-30 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Fell et al. (U.S. patent No. 5,202,238) in view of Casey et al. (June 2000, Protein Engineering Vol. 13 (6):445-452). Claims 1-9 and 11-14 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Fell et al. (U.S. patent No. 5,202,238) in view of Casey et al. (June 2000, Protein Engineering Vol. 13 (6):445-452) as applied to claims 20-21 and 27-30 and further in view of U.S. patent No. 6,570,061, Rajewsky et al. Applicants have amended the claims and have provided arguments to obviate this rejection. Thus, withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. §101

Claims 1-16 and 52 have been rejected under 35 U.S.C. §101. The Examiner alleges that the claimed invention is drawn to non-statutory subject matter. In particular, the claims recite genetically modified mammals, thereby including humans, which are not patentable. Applicants have amended the claims to recite genetically modified non-human mammals, thereby obviating the Examiner's rejection. Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. §112

Claims 1, 10, 11, 15-17, 26-27 and 31-32 have been rejected under 35 U.S.C. §112, first paragraph for non-compliance with the written description requirement. In particular, the Examiner alleges that the specification does not provide written description for proteins or polypeptides capable of quenching fluorescence, or for detectable proteins

that are a fusion between two different detectable markers, or specifically green fluorescent protein and alkaline phosphatase. The Examiner further alleges that the specification fails to disclose the nucleic acid sequences which encode these proteins for use in making the claimed genetically modified cells or mammals.

Applicants respectfully traverse the Examiner's rejection for the following reasons. The specification provides support for green fluorescent protein (GFP) being one of the preferred embodiments. In particular, support can be found in the specification on page 12, lines 9-12 for GFP of Aequorea victoria:

> "The green fluorescent protein of *Aequorea victoria* is particularly preferred as the fluorescent protein. A cDNA for the protein has been cloned (D. C. Prasher et al., "Primary structure of the *Aequorea victoria* green-fluorescent protein," Gene (1992) 111:229-33.)."

Further support for GFP can be found on page 13, lines 5-22, continuing on to page 14, lines 1-6. Reference is made to U.S. patent number 6,077, 707 which describes:

> "a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein but differs by at least a substitution at T203 and, in particular, T203X, wherein X is an aromatic amino acid selected from H, Y, W or F."

Furthermore, another preferred embodiment is red fluorescent protein. Support for this protein can be found in the specification on page 14, lines 10-20.

Support for written description of polypeptides capable of being detected by enzymatic activity can be found on page 14, line 22 and continuing on to page 15, lines 1-10.

Support for written description and guidance as to the selection of the immunoglobulin genes and locations therein in which to fuse the detectable polypeptides of the invention can be found on page 15, lines 18-21, continued on to page 16, lines 1-22 and on page 17, lines 1-13.

Given the guidance provided in the instant specification for the detectable proteins and the immunoglobulin genes, Applicants assert that one of skill in the art can utilize this information to produce a fusion polypeptide containing both the immunoglobulin genes with at least one detectable polypeptide gene. Furthermore, one of

skill in the art would be aware of the steps necessary to produce a fusion polypeptide containing two detectable polypeptide genes and an immunoglobulin gene, in tandem or not in tandem, optionally separated from the immunoglobulin portion of the polypeptide by one or more linker sequences and encoded by the polynucleotides provided in the instant application. As noted in the instant application, the two detectable polypeptides may be encoded by genes within the heavy or light chain constant regions.

Furthermore, support for fluorescent quenching proteins can be found in the reference provided on page 20, lines 7-15 of the instant application, in particular U.S. patent No. 5,998,204, which has been incorporated by reference in its entirety. A copy of this patent is also enclosed for the Examiner's convenience as part of Exhibit B. The Examiner's attention may be drawn specifically to column 6, lines 38-60; column 7, lines 11-15; column 9, lines 37-43; and column 10, lines 1-43; and also in column 11, lines 22-25. Accordingly, as stated in the reference incorporated herein, the fluorescent quenching proteins may be selected from any of those already cited in the present application. These proteins may be modified using the methods known to one skilled in the art and further as described in U.S. patent No. 5,998,204. Given the description provided therein, Applicants assert that one of skill in the art would be capable of synthesizing the genetic constructs provided in the present invention.

Withdrawal of the rejection is respectfully requested.

Claims 1-17, 26-27, 31-32 and 52 have been rejected under 35 U.S.C. 112, first paragraph for lack of enablement. Applicants respectfully traverse the Examiner's rejection, and have provided further support for enablement in a declaration under 37 C.F.R. 1.132, signed by Dr. Sanford Simon, with his curriculum vitae attached as Exhibit A. Furthermore, Applicants hereby assert that a person skilled in the art at the time of filing the present application would be knowledgeable in the preparation of transgenic animals and embryonic stem cells from species other than mice. For the Examiner's convenience, Applicants have provided several publications and abstracts that support methods for preparing non-human transgenic animals and embryonic stem cells, attached herein as Exhibit B. A few of the references have been supplied as abstracts, but the

entire papers have been ordered and will be submitted upon receipt. Accordingly, Applicants respectfully assert that it would not require undue experimentation at the time of filing for a skilled artisan to practice the invention. Withdrawal of the rejection is respectfully requested.

Furthermore, the Examiner alleges that in regard to claims 1, 10, 11, 15-17, 26-27 and 31-32, the specification does not provide enablement for a nucleic acid encoding a protein that quenches fluorescence or a chimeric detectable protein. With respect to the protein that quenches fluorescence, Applicants assert that U.S. patent No. 5,998,204, which was incorporated by reference in its entirety in the present application, teaches that any protein such as those described in the present application, in particular green fluorescent protein, can be modified using methods known to one skilled in the art and further as described in U.S. patent No. 5,998,204 to support enablement of the present invention. Furthermore, the claims have been amended to clarify that the chimeric antibody molecule contains two detectable proteins, optionally separated from the immunoglobulin protein by a linker protein. Withdrawal of the rejection is respectfully requested.

Claims 1-32 have been rejected under 35 U.S.C. 112, second paragraph as being indefinite. In particular, the Examiner alleges that independent claims 1 and 17 recite genetically modified immune cells or mammals capable of expressing a chimeric immunoglobulin gene "comprising at least one detectable protein or peptide fused with a gene expressing an immunoglobulin component". Applicants respectfully traverse the Examiner's rejection and have amended claims 1 and 17 to recite "a polynucleotide sequence encoding..." after the word "comprising" as suggested by the Examiner. Withdrawal of the rejection is respectfully requested.

Claims 11, 15-16, 27 and 31-32 have been rejected under 35 U.S.C. 112, second paragraph as being indefinite. In particular, the Examiner asserts that the recitation of "said at least one detectable protein is a combination of an autofluorescent protein or peptide and an enzymatically-active protein or polypeptide" Thus, it is unclear whether

the Applicant means that the combination is a single protein which is both autofluorescent and enzymatically active, or whether the chimeric immunoglobulin includes two separate detectable markers. The claims have been amended to better clarify what Applicants regard as the invention, that is, that the chimeric immunoglobulin includes two separate detectable markers, optionally separated from the immunoglobulin portion of the polypeptide by one or more linker sequences. Furthermore, the support for the amended claims can be found in the specification on page 15, lines 12-15; and on page 17, lines 2-5.

In addition, Applicants respectfully draw the Examiner's attention to the specification on page 11, lines 14-22, continuing on to page 12, lines 1-4; and to page 15, lines 17-21, continuing on to page 16, lines 1-11, which provide support for how the Applicants envision the insertion of the genes for the detectable protein markers into the immunoglobulin gene, thus providing a chimeric antibody molecule that contains at least one detectable protein marker. However, as noted in the specification on page 18, lines 9-17, one advantage of the present invention over the art cited is that a chimeric antibody may be prepared which may contain two different means of detection, for example, both a fluorescent marker as well as an enzyme marker, thus enabling the use of the antigen specific antibody for performing more than one function and thus may be applicable to various research and diagnostic applications, eg. the same chimeric antibody when generated with both a fluorescent label as well as an enzyme label may be used for immunohistological labeling of tissue sections, or for use for Western blot analysis or with a fluorescence activated cell sorter, or the same chimeric antibody when generated with both a fluorescent label and an intein that can be conjugated to a toxin or radiolabel can be used for tracking and therapeutic purposes.

Furthermore, to clarify the techniques envisioned by the Applicant, the Examiner's attention is also respectfully drawn to page 20, lines 17-22, continuing on to page 21, lines 1-11:

> "Such chimeric antibodies are prepared by following the methods described herein. In the example wherein the fluorescence is detected, the genes encoding the chains of the secreted immunoglobulin are modified at the polynucleotide level to provide that both a fluorescent peptide or polypeptide, and a fluorescence-

quenching or -modulating peptide or polypeptide, are fused into either the heavy immunoglobulin chain or the light immunoglobulin chain in the appropriate position in the genome of the mouse. The positions of integration into the respective immunoglobulin components are such that the proximity of the fluorescent polypeptide and the fluorescence-quenching or -modulating polypeptide in the secreted, whole immunoglobulin molecule, change on binding of the immunoglobulin to its target antigen. This change in proximity alters the interaction between the fluorophore and the quencher or modulator resulting in a modulation in the detectable fluorescence on exposure of the immunoglobulin to its excitation wavelength. Such positions are selected to not alter the ability of the complete immunoglobulin molecule to assemble, be secreted, or bind the antigen. These selections are within the realm of the skilled artisan. Known fluorescent peptides or polypeptides as well as peptides and polypeptides capable of quenching or modulating the fluorescence, such as FRET pairs, are known and can be selected to provide the chimeric immunoglobulin, and mammals capable of producing the immunoglobulin after immunization."

Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. §102

Claims 17-19 and 22-25 have been rejected under 35 U.S.C. 102(b) as being anticipated by Fell et al. in U.S. patent No. 5,202,238. Applicants respectfully traverse the Examiner's rejection for the following reasons.

Fell et al. teach genetically modified antibody producing cells which have undergone homologous recombination ***in vitro*** to replace a component of the immunoglobulin genes with all or a portion of a human variable or constant gene linked to an enzyme or substrate such as beta galactosidase, alkaline phosphatase or horseradish peroxidase. Fell et al teach that the antibodies produced by these cells can be used as labeled antibodies in diagnostic assays without further modification. Furthermore, Fell et al teach that the replacement gene can be inserted into either or both the light or heavy chain immunoglobulin genes. Furthermore, Fell et al. provide an embodiment whereby the replacement gene encodes all or a portion of IgG1, thus having a linked enzyme present in exon G1.

Applicants assert that the immune cells used for exposure to an antigen are prepared ***in vitro,*** a technique which oftentimes does not produce the desired effect in terms of the antibody produced. Applicants have identified a means for generation of a

detectable antibody specific for a preselected antigen ***in vivo,*** and have thus achieved a more accurate means of achieving the desired immune response. Thus, embryonic stem cells are first transfected with a targeting vector containing the immunoglobin region and the detectable protein region construct. These ES cells are then injected into blastocysts and implanted into female non-human mammals. The resulting chimeric non-human mammals are used to parent non-human mammals. Two heterozygous non-human mammals are then used to produce homozygous non-human mammals. Accordingly, upon injection of a preselected antigen, the non-human mammals will produce antibody specific for the antigen and will also contain the detectable protein. Thus, rather than engineering the antibody producing cells ***in vitro*** as demonstrated by Fell et al., Applicants have engineered the non-human mammals to produce the desired antibody producing cell ***in vivo***. Furthermore, as Fell et al. note in column 11, lines 50-55, the antibodies produced using their *in vitro* approach may be used for *in vivo* targeting of enzymes, toxins, drugs etc. **Fell et al. neither teach nor suggest production of the detectable chimeric antibodies *in vivo***. Furthermore, Fell et al. do not teach that the chimeric antibodies produced *in vitro* contain a flexible linker sequence between the immunoglobulin component and the detectable protein component. Applicants assert that the claims of the instant application, as currently amended, are not anticipated by Fell et al. Withdrawal of the rejection is respectfully requested.

Claim Rejections under 35 U.S.C. §103

Claims 20-21 and 27-30 are rejected under 35 U.S.C. §103 as being unpatentable over Fell et al. (U.S. patent No. 5,202,238) in view of Casey et al. (June 2000) Prot Engineer. Vol. 13(6):445-452. Furthermore, claims 1-9 and 11-14 have been rejected under 35 U.S.C. §103 as being unpatentable over Fell et al. (U.S. patent No. 5,202,238) in view of Casey et al. (June 2000) Prot Engineer. Vol. 13(6):445-452 as applied to claims 20-21 and 27-30, and further in view of U.S. patent No. 6,570,061 (2003), Rajewsky et al.

The Examiner has the initial burden of establishing a *prima facie* case of obviousness. A finding of obviousness under § 103 requires a determination of the scope

and content of the prior art, the differences between the claimed invention and the prior art, the level of ordinary skill in the art, and whether the differences are such that the claimed subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. Graham v. Deere, 383 US 1 (1966). Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion that the combination be made. In re Stencel, 828 F2d 751, 4 USPQ2d 1071 (Fed. Cir. 1987).

The invention as claimed. Claim 20 has been canceled, and the dependency of claim 21 has been amended to now depend from claim 17. Thus, the rejection of claims 20 and 21 in light of the cited references is now mooted. Claim 27 has been amended to read on the genetically-modified immune cell obtained from the genetically modified non-human mammal, which contains at least one detectable protein or peptide which is an autofluorescent protein or peptide, a visibly-detectable protein or peptide, an enzymatically active protein or peptide, a protein or peptide capable of interacting with another molecule to produce a detectable product, wherein said protein or peptide capable of interacting with another molecule to produce a detectable product is selected from the group consisting of an intein, a biotin-binding subunit of streptavidin or avidin, a His tag, a chitin-binding domain, or any combination thereof. The claims depending from claim 27 recite various detectable proteins which are encoded by the genes comprising the chimeric antibody.

The Fell et al. reference as a whole. As noted above, Fell et al teach genetically modified antibody producing cells which have undergone homologous recombination ***in vitro*** to replace a component of the immunoglobulin genes with all or a portion of a human variable or constant gene linked to an enzyme or substrate such as beta galactosidase, alkaline phosphatase or horseradish peroxidase. Fell et al teach that the antibodies produced by these cells can be used as labeled antibodies in diagnostic assays without further modification. Furthermore, Fell et al teach that the replacement gene can be inserted into either or both the light or heavy chain immunoglobulin genes. Furthermore,

Fell et al. provide an embodiment whereby the replacement gene encodes all or a portion of IgG1, thus having a linked enzyme present in exon G1.

Fell et al. do not disclose the preparation or production of genetically modified antibody producing cells ***in vivo***, nor do Fell et al disclose the possible variations of the detectable proteins envisioned by the present application, as now claimed in amended claim number 27. In addition, Fell et al. do not disclose a flexible linker present between the immunoglobulin region and the detectable protein region.

Casey et al. reference as a whole. Casey et al. describe the construction of a single chain antibody *in vitro* in a bacterial system. This antibody contains a flexible glycine linker and GFP.

Casey et al. do not teach or disclose the production of antibody producing cells *in vivo*, nor do Casey et al. teach or disclose the particular detectable proteins now claimed in amended claim number 27. Furthermore, as one skilled in the art can appreciate, the procedures for preparing an antibody molecule in a bacterial system does not allow for correct post-translational modification of the antibody molecule, such as glycosylation, which is needed for maximizing function and specificity.

Rajewsky et al. (U.S. patent No. 6,570,061) reference as a whole. Rajewsky et al. teach the use of homologous recombination to replace the constant region genes of the murine immunoglobulin heavy or light chain with human genes in murine embryonic stem cells and the use of these cells to make transgenic mice which produce the chimeric antibody.

Rajewsky et al do not teach the introduction of the detectable marker proteins into the chimeric immunoglobulin molecule as disclosed in the instant application, and as currently claimed. Nor do Rajewsky et al. teach an *in vivo* method of generating detectable labeled antibodies specific for a preselected antigen. In particular, Rajewsky et al. do not envision the potential for combining an enzyme label with a fluorescent label on the chimeric immunoglobulin molecule such that the antibodies so produced would serve multiple functions for research or diagnostic use.

The analysis under § 103(a). Fell et al. do not teach the preparation or production of genetically modified antibody producing cells ***in vivo***, nor do Fell et al disclose the possible variations of the detectable proteins envisioned by the present application, as now claimed in amended claim number 27. In addition, Fell et al. do not disclose a flexible linker present between the immunoglobulin region and the detectable protein region.

Casey et al. do not teach or disclose the production of antibody producing cells *in vivo*, nor do Casey et al. teach or disclose the particular detectable proteins now claimed in the present application.

Rajewsky et al do not teach the introduction of the detectable marker proteins into the chimeric immunoglobulin molecule as disclosed in the instant application, and as currently claimed. Nor do Rajewsky et al. teach an *in vivo* method of generating detectable labeled antibodies specific for a preselected antigen. In particular, Rajewsky et al. do not envision the potential for combining an enzyme label with a fluorescent label on the chimeric immunoglobulin molecule such that the antibodies so produced would serve multiple functions for research or diagnostic use.

Moreover, it was not until Applicants' present invention that the importance of the introduction of at least one detectable protein, and in certain cases, multiple detectable proteins was envisioned to allow for multiple *in vitro* diagnostic uses. Furthermore, it was not until the time of the present invention that an *in vivo* method for the production of genetically engineered non-human mammals that produce a chimeric antibody containing detectable proteins in response to a preselected antigen was possible. It has been 14 years since the date of filing of the Fell et al. application, and 12 years since the earliest filing of the Rajewsky et al. patent. Applicants assert that if it were obvious to combine the work of Fell et al., Casey et al. and Rajewsky et al., it would have been accomplished prior to the teachings of the present application. Accordingly, it is Applicants further assertion that these references do not teach or suggest the *in vivo* production of genetically modified non-human mammals that produce the chimeric antibodies having the characteristics described herein.

In fact, it was Applicants' own investigative work which identified the need for

production of a genetically engineered non-human mammal which would produce detectably labeled antibodies in response to antigenic challenge *in vivo*. Furthermore, Applicants own work recognized the benefits of creating a chimeric molecule which was engineered to have a variety of functions retained such as the ability to bind antigen, the ability to fluoresce such that the antibody can be used for diagnostic purposes, and the ability to secrete the labeled antibody without interfering with the membrane bound form of the antibody, which may inhibit targeting or processing of the protein. In addition, the Applicants of the present application recognized that one can use the genetically engineered non-human mammals repeatedly and with various antigenic challenges. It is Applicants assertion that none of the foregoing would be obvious in light of the cited references.

In light of the foregoing arguments and claim amendments, Applicants respectfully request withdrawal of the rejection.

Conclusion

Applicants believe that the foregoing amendments to the claims place the application in condition for allowance. Withdrawal of the rejections is respectfully requested. If a discussion with the undersigned will be of assistance in resolving any remaining issues, the Examiner is invited to telephone the undersigned at (201) 487-5800, ext. 118, to effect a resolution.

Respectfully submitted,

Veronica Mallon

Veronica Mallon, Ph. D.
Agent for Applicants
Reg. No. 52,491

KLAUBER & JACKSON
411 Hackensack Avenue
Hackensack, NJ 07601
Tel: (201) 487-5800

Attachments: Declaration under 37 CFR 1.132 signed by Dr. Sanford Simon
Curriculum vitae of Dr. Sanford Simon (Exhibit A)
Publications in support of enablement (Exhibit B)